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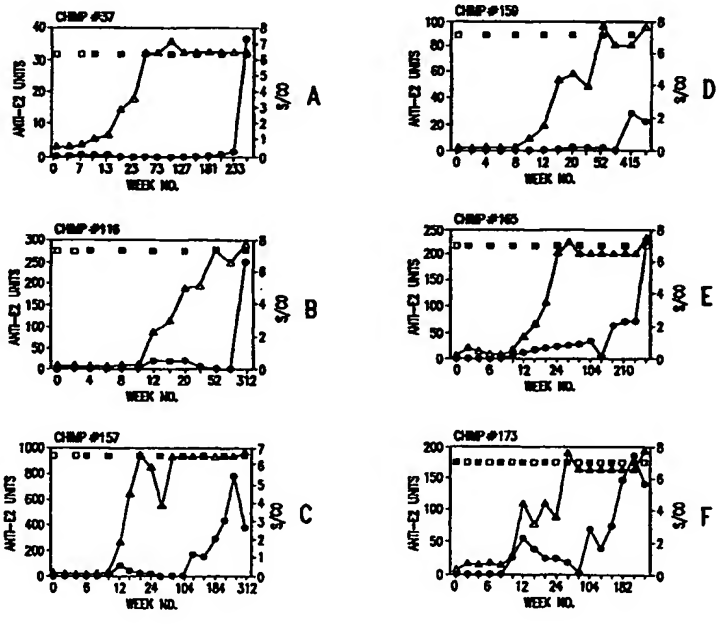

(57) Abstract

The subject invention relates to methods of detecting chronic infection in a patient who has been exposed to Hepatitis C virus. In particular, the present invention relates to methods of detecting antibody to the E2 protein of Hepatitis C virus in order to conclusively diagnosis chronic infection in a patient.

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METHODS OF DETECTING CHRONIC INFECTION CAUSED BY HCV

The subject application is a Continuation-In-Part of pending U.S. patent application serial no. 08/481,018, filed on June 7, 1995, which is hereby incorporated in its entirety by reference.

BACKGROUND OF THE INVENTION

Technical Field

The subject invention relates to methods of detecting chronic infection in a patient who has been exposed to Hepatitis C virus. In particular, the present invention relates to methods of detecting antibody to the E2 protein of Hepatitis C virus in order conclusively diagnose chronic infection in a patient.

Background Information

Hepatitis C virus (HCV) is a major cause of human morbidity and mortality. Between 20 and 50% of infections caused by this virus are self-limited; however, the remainder of the infections progress to chronic infections. Patients with such chronic infections have a high risk of development of cirrhosis and hepatocellular carcinoma. There are an estimated 500 million chronic carriers in the world today. Thus, it is important to have the ability to diagnose the self-limited form of the infection, which requires no further treatment, and to distinguish this form from the onset of chronic infection, which may require treatment.

HCV has a single-stranded genome which codes for a polyprotein of approximately 3000 amino acids comprising,

successively, the capsid, the envelope proteins E1 and E2, and then the non-structural proteins NS2, NS3, NS4 a and b, and NS5 a and b. The E2 protein has been of particular interest since its hypervariable region appears to vary under immune selection and may therefore be the site of epitopes reacting with neutralizing antibodies (Weiner et al., Proc. Natl. Acad. Sci. USA 89:3468-72 (1992); Kato et al., J. Virol. 67:3923-30 (1993); Taniguchi et al., Virology 195:297-301 (1993)). The E2 antigen has been expressed in baculovirus expression systems and in mammalian cells (Matsuura et al., Intervirology 37:114-118 (1994); Dubuisson et al., J. Virol. 68:6147-60 (1994); Matsuura et al., Virology 205:141-50 (1994); Lesniewski et al., J. Med. Virol. 45:415-22 (1995)). However, the latter appear to give the highest reactivity with patient sera.

In the first report using mammalian derived E2 for analysis of patient sera, Zaaijer et al. found that this assay was useful in resolving cases with indeterminate Riba-2 results (Zaaijer et al., J. Med. Virol. 44:395-97 (1994)). Twenty-nine of 33 Rib indeterminate PCR(+) sera had anti-E2 detectable by enzyme-linked immunosorbent assay, as compared to only 2 of 39 PCR(-) sera. Leon et al. confirmed these results and suggested that E2 should be added to the antigens found in polyvalent screening assays (Leon et al., Vox Sang 70:213-16 (1996)). Yuki et al. found that the prevalence of anti-E2 was related to levels of viremia, such anti-E2 being higher in those patients with high levels of circulating HCV PCR reactivity (Yuki et al., Hepatology 23:947-52 (1996)). These authors suggested that their results did not support effective neutralization by anti-E2.

Fornillier-Jacob et al., using an indirect immunofluorescence assay for detection of anti-E2 antibody, noted a potential relationship between prevalence of anti-E2 and future possible chronicity (Fornillier-Jacob et al., J. Med. Virol. 50:159-67 (1996)). Lee et al. found that anti-E2 was frequently positive in otherwise seronegative chronically HCV infected immunocompromised hemodialysis patients (Lee et a., J. Am. Soc. Nephrol. 7:2409-13 (1996)). Grellier et al. determined the presence or absence of anti-E2 antibody in a cohort of 87 women infected with HCV from contaminated anti-D immunoglobulin (Grellier et al., J. Viral Hepatitis 4:379-381 (1997)). In particular, anti-E2 antibody was found in 16 cases (100%) which were RNA positive, but in only 50 cases (62%) that were recombinant immunoblot (RIBA) positive but RNA negative. In the remaining 21 cases of women who were recombinant immunoblot indeterminate and RNA negative, anti-E2 antibody was found in only 3 cases (14%). The studies by Yuki et al., Fornillier-Jacob et al., Lee and Grellier et al. involved single-point testing of individual case samples at only one point in time or at only a few points in time. The studies did not establish a linkage between sustained high antibody titers to HCV E2 over time and chronic disease. In contrast, the present invention and data related thereto conclusively demonstrate the association between antibody production to HCV E2 and chronic disease. This was established by following a number of cases (chimpanzee animal model and infected humans) from the point of seroconversion through the course of illness leading either to resolution of disease associated with loss of anti-E2 titer and RNA detection by PCR, or chronic disease associated with sustained anti-E2

titers and RNA positivity. The data from the seroconversion cases suggest a prognostic and diagnostic value to monitoring antibody levels to E2, to be described in detail below.

It should be noted that there are different types of assays which may be utilized to measure or detect viral antigens or antibodies to such antigens. In particular, immunoassays have been developed with many different formats, but they may be divided into two main classes: 1) competitive assays and 2) non-competitive assays (e.g., immunometric, sandwich). For heterologous immunoassays of both classes, solid-phase biochemistry for separation of bound and free reactants has proven to be revolutionary. In particular, antibody or antigen reagents can be covalently or non-covalently (e.g., ionic, hydrophobic, etc.) attached to the solid phase. Linking agents for covalent attachment are known and may be part of the solid phase or derivatized to it prior to coating. Examples of solid phases used in immunoassays are porous and non-porous materials, latex particles, magnetic particles, microparticles, beads, membranes, microtiter wells and plastic tubes. The choice of solid phase material and method of labeling the antigen or antibody reagent is determined based on desired assay format performance characteristics. For some immunoassays, no label is required. For example, if the antigen is on a detectable particle such as a red blood cell, reactivity can be established based on agglutination. Alternatively, antigen-antibody reaction may result in a visible change (e.g., radial immunodiffusion). In most cases, one of the antibody or antigen reagents used in an immunoassay is attached to a signal generating compound or "label". This

signal generating compound or "label" is in itself detectable or may be reacted with one or more additional compounds to generate a detectable product. Examples of signal generating compounds include chromogens, radioisotopes (e.g., 125I, 131I, 32P, 3H, 35S and 14C), fluorescent compounds, particles (visible or fluorescent), nucleic acids, complexing agents, or catalysts such as enzymes (e.g., alkaline phosphatase, acid phosphatase, horseradish peroxidase, beta-galactosidase, and ribonuclease). In the case of enzyme use, addition of chromo-, fluoro-, or lumo-genic substrate results in generation of a detectable signal. Other detection systems such as time-resolved fluorescence, internal-reflection fluorescence, amplification (e.g., polymerase chain reaction) and Raman spectroscopy are also useful.

Immunoassays have, in fact, been developed to monitor biological fluids (e.g., plasma, serum, cerebrospinal fluid, saliva, tears, nasal washes, or aqueous extracts of tissues and cells) for the presence of antibody specific for an antigen of interest (e.g., infectious agent such as HCV, auotoantigen or allergen). In many cases, these specific antibody immunoassays have been designed to be antibody class or subclass-specific. There are two general formats commonly used to monitor specific antibody in humans: 1) antigen is presented on a solid phase, the human biological fluid containing specific antibodies is allowed to react with the antigen, and then antibody bound to antigen is detected with an anti-human antibody coupled to a signal generating compound and 2) an anti-human antibody is bound to the solid phase, the human biological fluid containing specific antibodies is allowed to react with the antibody, and then antigen attached to a signal generating

compound is added to detect specific antibody. In both formats, the anti-human antibody reagent may be polyclonal or monoclonal. Morever, the anti-human antibody reagent may recognize all antibody classes, or alternatively, may be specific for a particular class or subclass of antibody depending on the intended purpose of the assay. Immunoassays designed to detect specific antibody provide a measure of antibody activity. This may be referred to as antibody titer, e.g., mid-point or end-point titer, or expressed in units (activity or gravimetric) relative to a reference standard. Such antibody assays will be described in detail below.

All U.S. patents and publications are herein incorporated in their entirety by reference.

SUMMARY OF THE INVENTION

The present invention encompasses a method of detecting chronic infection in a patient who has been exposed to Hepatitis C Virus (HCV) comprising the steps of: (a) contacting a test sample suspected of containing anti-E2 antibody with antigen specific for the antibody for a time and under conditions sufficient to allow the formation of antigen/antibody complexes; (b) detecting the amount of antibody which may be present in the test sample; and (c) correlating high titer or a sustained antibody titer to E2, as compared to at least one previous test result, with a diagnosis of chronic infection.

Additionally, the present invention encompasses a method of detecting chronic infection in a patient who has been exposed to HCV comprising the steps of: (a) contacting a test sample suspected of containing anti-E2 antibody with antigen specific for the antibody for a time and under

conditions sufficient to allow the formation of antigen/antibody complexes; (b) adding a conjugate to the resulting antigen/antibody complexes for a time and under conditions sufficient to allow the conjugate to bind to bound antibody, wherein the conjugate comprises an antibody attached to a signal generating compound capable of generating a detectable signal; (c) detecting the amount of antibody which may be present in said test sample, in comparison to a reference standard, by detecting the signal generated by the signal generating compound; and (d) correlating high titer or a sustained antibody titer to E2, as compared to at least one previous test result, with a diagnosis of chronic infection.

The present invention also includes a method for detecting chronic infection in a patient exposed to HCV comprising the steps of: (a) contacting a test sample suspected of containing anti-E2 antibody with anti-antibody specific for the antibody for a time and under conditions sufficient to allow the formation of anti-antibody/antibody complexes; (b) detecting the amount of antibody which may be present in the test sample; and (c) correlating high titer or a sustained antibody titer to E2, as compared to at least one previous test result, with a diagnosis of chronic infection.

Additionally, the present invention encompasses a method for detecting chronic infection in a patient exposed to HCV comprising the steps of: (a) contacting a test sample suspected of containing anti-E2 antibody with anti-antibody specific for the anti-E2 antibody for a time and under conditions sufficient to allow the formation of anti-antibody/antibody complexes; (b) adding a conjugate to resulting anti-antibody/antibody complexes for a time and

under conditions sufficient to allow the conjugate to bind to the bound antibody, wherein the conjugate comprises an antigen attached to a signal generating compound capable of generating a detectable signal; (c) detecting the amount of antibody which may be present in the sample in comparison to a reference standard, by detecting the signal generated by the signal generating compound; and (d) correlating high titer or a sustained antibody titer to E2, as compared to at least one previous test result, with a diagnosis of chronic infection.

Furthermore, the invention includes a method of detecting chronic infection in a patient who has been exposed to HCV comprising the steps of: (a) contacting a test sample suspected of containing anti-E2 antibody with at least one antigen specific for the antibody, for a time and under conditions sufficient to allow the formation of antigen/antibody complexes; (b) adding at least one conjugate to the antigen/antibody complexes for a time and under conditions sufficient to allow said at least one conjugate to bind to the bound antibodies, wherein the at least one conjugate comprises an antibody attached to a signal generating compound capable of generating a detectable signal; (c) detecting the amount of antibody which may be present in the test sample, in comparison to a reference standard, by detecting the signal generated by the signal generating compound; and (d) correlating high titer or a sustained antibody titer to E2, as compared to at least one previous test result, with a diagnosis of chronic infection.

Moreover, the present invention also includes a method of differentiating chronic infection from resolving (i.e., self-limiting) infection in a patient who has been

exposed to HCV comprising the steps of: (a) contacting a test sample suspected of containing anti-E2 antibody with antigen specific for the antibody for a time and under conditions sufficient to allow the formation of antigen/antibody complexes; (b) detecting the amount of antibody which may be present in the test sample; and (c) correlating high titer or a sustained antibody titer to E2, as compared to at least one previous test result, with a diagnosis of chronic infection and correlating a decrease or lack of antibody, as compared to at least one previous test result, with a diagnosis of a resolving HCV infection.

Furthermore, the present invention includes a sandwich method of detecting chronic infection in a patient who has been exposed to HCV comprising the steps of: (a) contacting a test sample suspected of containing anti-E2 antibody with a first antigen specific for said antibody for a time and under conditions sufficient to allow the formation of antigen/antibody complexes; (b) adding a conjugate to the resulting antigen/antibody complexes of step (a) for a time and under conditions sufficient to allow said conjugate to bind to bound antibody, wherein said conjugate comprises a second antigen attached to a signal generating compound capable of generating a detectable signal; and (c) detecting the amount of antibody which may be present in the test sample, in comparison to a reference standard, by detecting the signal generated by the signal generating compound; and (d) correlating high titer or a sustained antibody titer to E2, as compared to at least one previous test result, with a diagnosis of chronic infection.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 illustrates the serial follow-up of chimpanzees developing chronic HCV infection (large rectangles: RT PCR (filled = positive, empty = negative; curve with empty triangles: anti-HCV 3.0; curve with filled circle: anti-E2).

Figure 2 represents the serial follow-up of chimpanzees developing self-limited HCV infection (symbols as in Figure 1).

Figure 3 represents the serial follow-up of patients developing chronic HCV infection (symbols as in Figure 1).

Figure 4 represents a serial follow-up of patients developing self-limited HCV infection (symbols as in Figure 1).

DETAILED DESCRIPTION OF THE INVENTION

The present invention relates to methods of detecting chronic infection in mammalian patients who have been exposed to HCV. In particular, these methods are based upon the discovery that chimpanzees and humans having chronic infection show a higher frequency of development of antibodies to E2 and higher E2 antibody titer than those having a self-limited infection. As stated above, the HCV E2 protein has been of considerable interest as a target for immunotherapy since its hypervariable region varies under immune selection; thus, it has been thought to be the site of epitopes reacting with neutralizing antibodies. However, similar to the case of Human Immunodeficiency Virus, it has been difficult to demonstrate efficacy of antibody neutralization in HCV disease.

As evidenced by the data presented in the examples below, anti-E2 antibodies do not play a role in the mechanism of self-limitation of HCV infections, as might

have been expected. In fact, anti-E2 antibodies develop earlier, more frequently, and to a higher titer in chimpanzees and in humans developing chronic infection than in those with self-limited infections. Thus, antibodies against E2 are unlikely to play a role in self-limiting infection; however, long-term persistence of antibodies to E2 correlates with chronic infection. Absence of a persisting antibody response correlates with resolution of the infection.

In view of the above findings, the present invention encompasses a method of detecting chronic infection in a patient who has been exposed to HCV. This method comprises the steps of (a) contacting a test sample suspected of containing the anti-E2 antibody with antigen specific for the antibody for a time and under conditions sufficient to allow the formation of antigen/antibody complexes; (b) detecting the amount of antibody which may be present in the test sample; and (c) correlating high titer or a sustained antibody titer to E2, as compared to at least one previous test result, with a diagnosis of chronic infection. For example, from the results described below, among the chronically-infected chimpanzee cases (based upon an average RNA positive duration of approximately > 300 weeks), the average point of anti-E2 seroconversion was 120 weeks with an average peak anti-E2 titer of 252 units. Four out of six of these cases showed anti-E2 seroconversion as early as 10-16 weeks following infection, and these individuals ultimately developed an average anti-E2 titer of approximately 357 units. Among the chimpanzee cases with resolving disease (average RNA positive duration of <16 weeks), four out of six did not seroconvert to anti-E2 positivity even >200 weeks post infection. Two out of 6

did seroconvert 11-12 weeks post-infection, but these individuals only ultimately developed an average anti-E2 titer of approximately 107 units. Among the chronically-infected humans (average RNA positive duration 156 weeks), anti-E2 seroconversion occurred 4-11 weeks post-infection, and these cases developed an ultimate average anti-E2 titer of approximately 1443 units. Among the human cases with resolving disease (average RNA positive duration 8 weeks), seroconversion to anti-E2 positivity occurred from 12-104 weeks post infection; however, average anti-E2 peak titer was only approximately 277 units, and antibody did not develop at all in two of these cases.

In order to definitively establish a "cutoff value" for anti-E2 titer, showing a high correlation with chronic HCV disease from a single point determination, would require the testing of many additional cases of known chronic and self-limiting disease. However, from the data presented below, it can be observed that chimpanzees with chronic disease developed approximately a 2.4-fold higher anti-E2 titer than did those with resolving disease. While six out of six chronically-infected chimpanzees developed anti-E2, only two out of six resolving cases developed anti-E2. Anti-E2 positive humans with chronic disease developed three-fold higher average anti-E2 titers than did those with resolving disease. Two out of the 5 resolving cases never developed anti-E2. Thus, relatively higher and more sustained anti-E2 titers are definitively associated with chronic disease.

The cases with resolving disease often showed early transient or fluctuating titers to E2. Such an observation suggests that, from a prognostic standpoint, it would be appropriate to monitor antibody levels in infected cases on

an ongoing basis, e.g., monthly to track changes in titer. Development of a sustained anti-E2 response would signal a propensity to develop chronic disease while fluctuation, lack or loss of anti-E2 antibody would correlate with a tendency to resolve infection.

The quantitation of antibody to E2 may be accomplished by measuring the antibody titer, by comparison to a known control sample from an uninfected individual (as a negative control), or by assigning a unit value of antibody to the test sample by taking the test sample dilution multiplied by the signal output in the test immunoassay. For example, in an Enzyme Linked Immunosorbent Assay (ELISA) employing a chromogenic substrate, the signal output would equate to the optical density (O.D.) value of the neat or diluted test sample at the conclusion of the assay. A reference curve could be constructed using a pool of known seropositive samples to establish the linear range over which this approach would yield the best accuracy. The test sample could be, for example, plasma, serum or whole blood. The means of reliably detecting and quantitating the titer or quantity of antibody to E2 should be readily apparent to those of ordinary skill in the art.

Additionally, the present invention includes another method of detecting chronic infection in a patient who has been exposed to HCV comprising the steps of (a) contacting a test sample suspected of containing the anti-E2 antibody with antigen specific for the antibody for a time and under conditions sufficient to allow the formation of antigen/antibody complexes; (b) adding a direct or indirect conjugate to the resulting antigen/antibody complexes for a time and under conditions sufficient to allow the conjugate to bind to the bound antibody, wherein the conjugate

comprises an antibody attached to a signal generating compound capable of generating a detectable signal; and (c) detecting the amount of antibody which may be present in the test sample, in comparison to a reference standard, by detecting the signal generated by the signal generating compound; and (c) correlating high titer or a sustained antibody titer to E2, as compared to at least one previous test result, with a diagnosis of chronic infection.

Additionally, the present invention includes a method for detecting chronic infection in a patient exposed to HCV comprising the steps of (a) contacting a test sample suspected of containing anti-E2 antibody with anti-antibody specific for the antibody for a time and under conditions sufficient to allow the formation of anti-antibody/antibody complexes; (b) detecting the amount of antibody which may be present in the test sample; and (c) correlating high titer or a sustained antibody titer to E2, as compared to at least one previous test result, with a diagnosis of chronic infection.

Furthermore, the present invention also encompasses a method for detecting chronic infection in a patient exposed to HCV comprising the steps of (a) contacting a test sample suspected of containing anti-E2 antibody with anti-antibody specific for the anti-E2 antibody for a time and under conditions sufficient to allow the formation of anti-antibody/antibody complexes; (b) adding a conjugate to the resulting anti-antibody/antibody complexes for a time and under conditions sufficient to allow the conjugate to bind to the bound antibody, wherein the conjugate comprises an antigen attached to a signal generating compound capable of generating a detectable signal; (c) detecting the amount of antibody which may be present in the test sample in

comparison to a reference standard, by detecting the signal generated by the signal generating compound; and (d) correlating high titer or a sustained antibody titer to E2, as compared to at least one previous test result, with a diagnosis of chronic infection.

Moreover, the present invention also includes a method of detecting chronic infection in a patient who has been exposed to HCV comprising the steps of: (a) contacting a test sample suspected of containing antibodies to anti-E2 antibody with at least one antigen specific for these antibodies, for a time and under conditions sufficient to allow the formation of antigen/antibody complexes; (b) adding at least one conjugate to said antigen/antibody complexes for a time and under conditions sufficient to allow the at least one conjugate to bind to the bound antibodies, wherein the at least one conjugate comprises an antibody attached to a signal generating compound capable of generating a detectable signal; (c) detecting the amount of antibody which may be present in the test sample, in comparison to a reference standard, by detecting the signal generated by the signal generating compound; and (d) correlating high titer or a sustained antibody titer to E2, as compared to at least one previous test result, with a diagnosis of chronic infection.

Additionally, the present invention also includes a method to differentiate resolving HCV disease from chronic HCV disease. The lack of or decrease (i.e., loss) of anti-E2 antibody titer correlates with resolution of infection and loss of detectable HCV viral RNA. In particular, this method of differentiation may comprise the steps of: (a) contacting a test sample suspected of containing the anti-E2 antibody with antigen specific for the antibody for

a time and under conditions sufficient to allow the formation of antigen/antibody complexes; (b) detecting the amount of antibody which may be present in the test sample; and (c) correlating high titer or a sustained antibody titer to E2, as compared to at least one previous test result, with a diagnosis of chronic infection and correlating a decrease or lack of antibody, as compared to at least one previous test result, with a diagnosis of a resolving HCV infection. As HCV has a propensity for chronicity, leading to significant morbidity, in later life (through, for example, the development of cirrhosis), the diagnosis of a resolving infection would certainly give psychological relief to the test patient. Furthermore, the diagnosis or prognosis of chronicity, based upon the methods of the present invention, would "earmark" the test patient exhibiting high or sustained antibody titers for more aggressive therapies. The methods of the present invention are certainly preferable to, for example, viral RNA detection by PCR, the latter of which is fraught with problems of reproducibility, specificity, and significant expense.

The present invention may be illustrated by the use of the following non-limiting examples:

EXAMPLE I

Serological Assay for Antibodies to E2

Patients: The patients studied were enrolled in a prospective post-transfusion follow up studied carried out in 1969-1973 (Prince et al., Lancet ii:241-246 (1974)). Twelve follow-up sera were obtained over a 9 month period. The sera had been stored at -70°C since that time.

Chimpanzees: The chimpanzees studied were housed at Vilab II, the New York Blood Center's chimpanzee research facility in Robertsfield, Liberia. Animals were housed in groups of at least two animals and resocialized into larger groups at the completion of protocols. The animals studied had been enrolled in various experiments, mostly utilizing HCV genotype 1b and were mostly followed after the acute infection for 5-10 years. All sera were stored at -70°C.

Assay: Mammalian cell-derived E2 protein was prepared and purified as described in Lesniewski et al. (J. Med. Virol. 45:415-22 (1995)). Purified E2 protein was coated onto polystyrene beads at 1.5 ug/ml. A semi-quantitative EIA was developed for measurement of anti-E2 in test human or chimpanzee samples. An anti-E2 unit value was assigned to each test sample by reference to a standard calibration curve generated with a pool of three known anti-E2 positive plasmas. All chimpanzee samples were tested undiluted; human samples were tested undiluted or retested at a 1:20 dilution if they were initially above the reference curve limits in the undiluted assay. Testing for anti-HCV antibody was carried out using a commercially available HCV antibody assay (HCV 3.0 EIA (Abbott Laboratories, N. Chicago, IL); Matrix HCV 2.0 (Abbott Laboratories) which was also used to assist in determining when seroconversion to active HCV antibody positive status occurred.

EXAMPLE II

Polymerase Chain Reaction (PCR) Assays

Procedure: Quantitative HCV DNA assays were carried out using the AmpliSensor assay system (Biotronics, Lowell, MA). The AmpliSensor assay system monitors the amplification efficiency of the PCR reaction via a fluorescence resonance energy transfer (FRET) based detection scheme (Wang et al., PCR Primer: A Laboratory Manual, New York: Cold Spring Harbor Press 1995:193-202). HCV RNA was reverse-transcribed within 5' untranslated region (5' UTR). The cDNA was amplified in an asymmetric manner to generate a 211 nucleotide long single strand target DNA. The single strand products were re-amplified in a semi-nested manner with the fluorescent primer duplex yielding a 66 bp amplicon. The in vitro transcript of $pHCV_{324}$ (Biotronics, Lowell, MA) was used as a standard for quantitation. Serial dilutions of this transcript were run as a standard in duplicate in every run. The Accugene system is based on serial fluorescence measurements carried out between the 26^{th} and 41^{st} PCR cycle. Thus, microplates were sealed throughout the cycling procedure and never opened after the 25^{th} cycle when fluorescent primers were added. This contributes to the control of contamination. PCR set up was carried out in a laminar flow hood in a dedicated room from which plasmids and amplicons are excluded.

Results: Figure 1 summarizes the 6 cases of HCV infection in chimpanzees which developed chronic infection as assessed by PCR. A rapid and strong anti-HCV antibody response was seen in all animals. Anti-HCV E2 antibody

responses occurred in all animals, although in two cases, this occurred late in follow-up. Times of seroconversion in these and the other cases studied are summarized in Table 1 below.

TABLE 1: ANTI-E2 SEROCONVERSION IN HCV INFECTIONS

CASES	CHIMP #	E2 SERCON. (WEEKS)	PEAK E2 UNITS	PEAK E2 UNITS (WEEKS)	ABBOTT 3.0 SEROCON. (WEEKS)	DURATION PCR (+) (WEEKS)
CHRONIC CHIMPS	37	259	36.5	259	11	>259
	116	12	247	312	12	>212
	157	10	783	232	12	>312
	159	415	29.5	415	12	>416
	165	16	233	312	12	>312
	173	10	186	212	10	>298
SELF-LIMTED CHIMPS	147	>416	0	None	12	16
	275	>360	0	None	10	8
	213	12	83.7	12	49	<12
	235	>208	0	None	52	<4
	238	11	131.2	11	20	<16
	266	>416	0	None	10	12
CHRONIC HUMANS	35	11	527	156	11	>156
	171	4	1902	78	4	>78
	184	11	1902	78	11	>78
SELF-LIMITED HUMANS	4	17	395	17	17	8
	67	>23	0	None	16	6
	123	>48	0	None	12	4
	155	104	56.9	104	10	8
	201	12	936	26	12	14

Chimpanzees which developed self-limited infection are summarized in Figure 2. Four of these animals did not develop anti-E2 despite many years of follow-up. The remaining 2 animals in this group had only early transitory anti-E2 responses. Anti-HCV total antibody responses in these animals, by contrast, were rapid, strong and did not differ from the animals which developed chronic infection (measured by HCV EIA 3.0).

Figures 3 and 4 summarize the human cases. These cases were more difficult to analyze than the chimpanzee cases in that most (Cases #35, 4, 67, 201 and 155) showed passive anti-HCV antibody resulting from the large number of transfusions received (mean=18) and the poor donor selection due to the unavailability of an anti-HCV screening assay at the time these patients were transfused (Prince et al., Lancet ii:241-246 (1974)). The cases developing chronic infection (see Figure 3) all developed strong anti-E2 responses. In two cases, these were rapid. Two of the cases developing self-limited infections (see Figure 4) did develop anti-E2 antibody, though more slowly than the chronic cases ($P<0.05$) and to lower peak titer ($P<0.05$). Two of these patients developed no detectable anti-E2 antibody.

CLAIMS:

1. A method of detecting chronic infection in a patient who has been exposed to Hepatitis C virus (HCV) comprising the steps of:

(a) contacting a test sample suspected of containing anti-E2 antibody with antigen specific for said antibody for a time and under conditions sufficient to allow the formation of antigen/antibody complexes;

(b) detecting the amount of antibody which may be present in said test sample; and

(c) correlating high titer or a sustained antibody titer to E2, as compared to at least one previous test result, with a diagnosis of chronic infection.

2. A method of detecting chronic infection in a patient who has been exposed to HCV comprising the steps of:

(a) contacting a test sample suspected of containing anti-E2 antibody with antigen specific for said antibody for a time and under conditions sufficient to allow the formation of antigen/antibody complexes;

(b) adding a direct or indirect conjugate to the resulting antigen/antibody complexes for a time and under conditions sufficient to allow the conjugate to bind to bound antibody, wherein said

conjugate comprises an antibody attached to a signal generating compound capable of generating a detectable signal; and

(c) detecting the amount of antibody which may be present in said test sample, in comparison to a reference standard, by detecting the signal generated by said signal generating compound; and

(d) correlating high titer or a sustained antibody titer to E2, as compared to at least one previous test result, with a diagnosis of chronic infection.

3. A method for detecting chronic infection in a patient exposed to HCV comprising the steps of:

(a) contacting a test sample suspected of containing anti-E2 antibody with anti-antibody specific for said antibody for a time and under conditions sufficient to allow the formation of anti-antibody/antibody complexes;

(b) detecting the amount of antibody which may be present in said test sample; and

(c) correlating high titer or a sustained antibody titer to E2, as compared to at least one previous test result, with a diagnosis of chronic infection.

4. A method for detecting chronic infection in a patient exposed to HCV comprising the steps of:

(a) contacting a test sample suspected of containing anti-E2 antibody with anti-antibody specific for said anti-E2 antibody for a time and under conditions sufficient to allow the formation of anti-antibody/antibody complexes;

(b) adding a conjugate to resulting anti-antibody/antibody complexes for a time and under conditions sufficient to allow the conjugate to bind to the bound antibody, wherein said conjugate comprises an antigen attached to a signal generating compound capable of generating a detectable signal;

(c) detecting the amount of antibody which may be present in said test sample in comparison to a reference standard, by detecting the signal generated by said signal generating compound; and

(d) correlating high titer or a sustained antibody titer to E2, as compared to at least one previous test result, with a diagnosis of chronic infection.

5. A method of detecting chronic infection in a patient who has been exposed to HCV comprising the steps of:

(a) contacting a test sample suspected of containing anti-E2 antibody with at least one antigen

specific for said antibody, for a time and under conditions sufficient to allow the formation of antigen/antibody complexes;

(b) adding at least one conjugate to said antigen/antibody complexes for a time and under conditions sufficient to allow said at least one conjugate to bind to said bound antibodies, wherein said at least one conjugate comprises an antibody attached to a signal generating compound capable of generating a detectable signal;

(c) detecting the amount of antibody which may be present in said test sample, in comparison to a reference standard, by detecting the signal generated by the signal generating compound; and

(d) correlating high titer or a sustained antibody titer to E2, as compared to at least one previous test result, with a diagnosis of chronic infection.

6. The method of claims 2, 4 or 5 wherein said conjugate comprises a chromogen, a fluorescent compound, a radioisotope, a particle, a nucleic acid, a catalyst and a complexing agent.

7. A method of differentiating chronic infection from resolving infection in a patient who has been exposed to HCV comprising the steps of:

(a) contacting a test sample suspected of containing anti-E2 antibody with antigen specific for said antibody for a time and under conditions sufficient to allow the formation of antigen/antibody complexes;

(b) detecting the amount of antibody which may be present in said test sample; and

(c) correlating high titer or a sustained antibody titer, as compared to at least one previous test result, with a diagnosis of chronic infection and correlating a decrease or lack of antibody, as compared to at least one previous test result, with a diagnosis of a resolving HCV infection.

8. A method of detecting chronic infection in a patient who has been exposed to HCV comprising the steps of:

(a) contacting a test sample suspected of containing anti-E2 antibody with a first antigen specific for said antibody for a time and under conditions sufficient to allow the formation of antigen/antibody complexes;

(b) adding a conjugate to the resulting antigen/antibody complexes of step (a) for a time and under conditions sufficient to allow said conjugate to bind to bound antibody, wherein said conjugate comprises a second antigen attached to a signal generating compound capable of generating a detectable signal; and

(c) detecting the amount of antibody which may be present in said test sample, in comparison to a reference standard, by detecting the signal generated by said signal generating compound; and

(d) correlating high titer or a sustained antibody titer to E2, as compared to at least one previous test result, with a diagnosis of chronic infection.

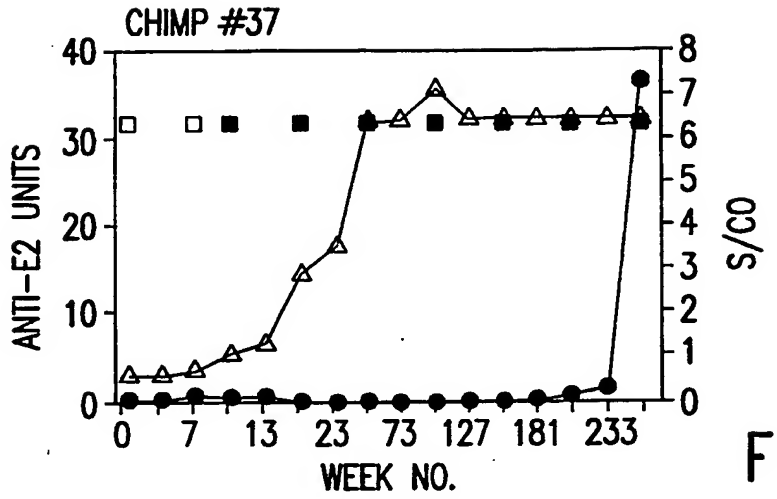

FIG.1A

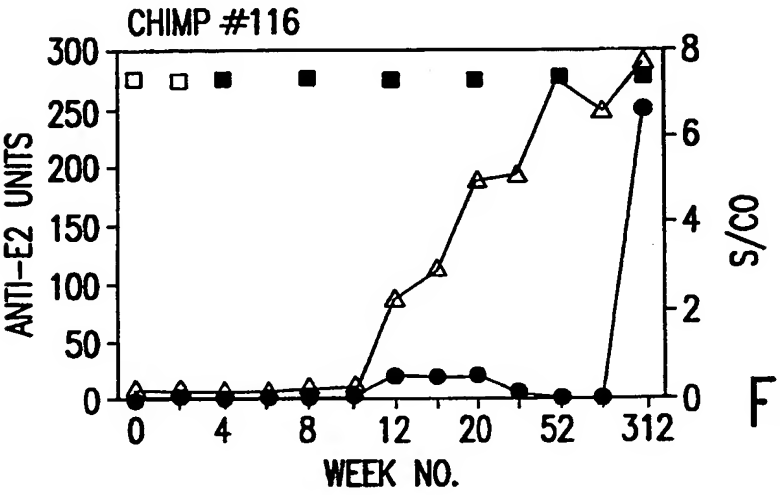

FIG.1B

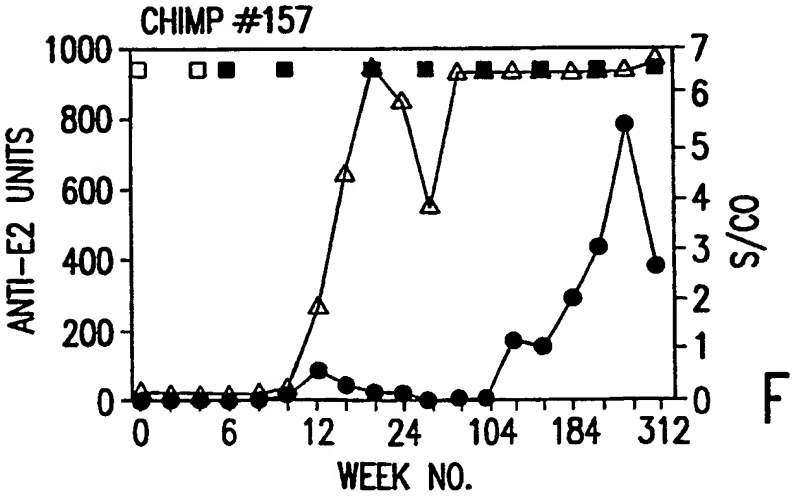

FIG.1C

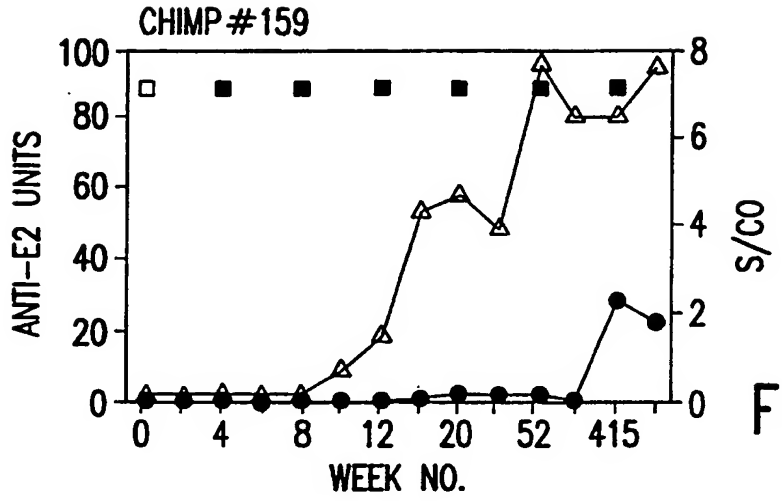

FIG.1D

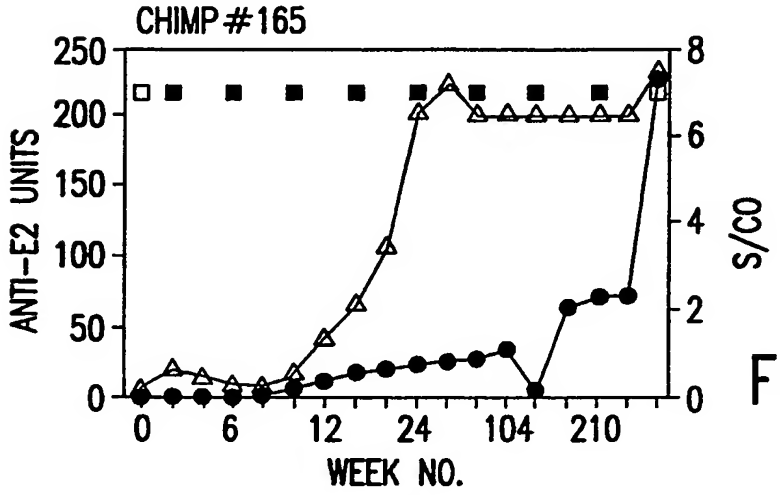

FIG.1E

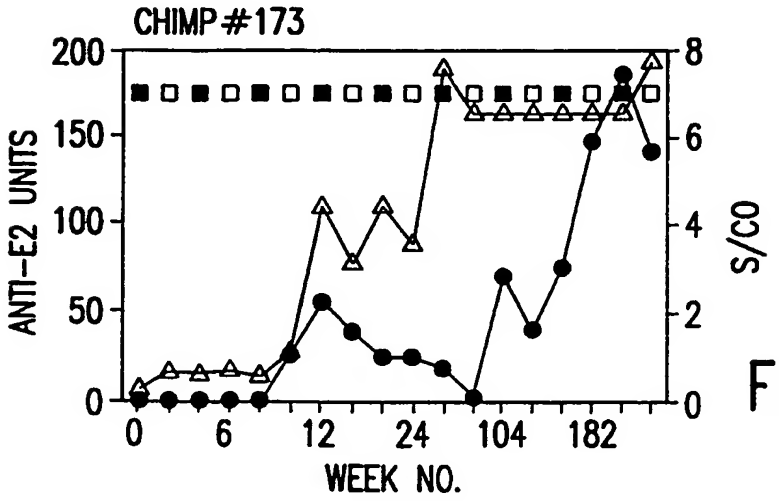

FIG.1F

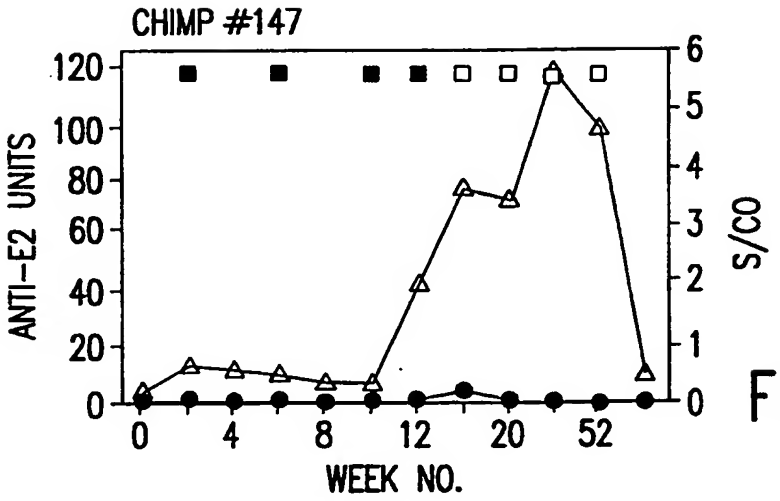

FIG.2A

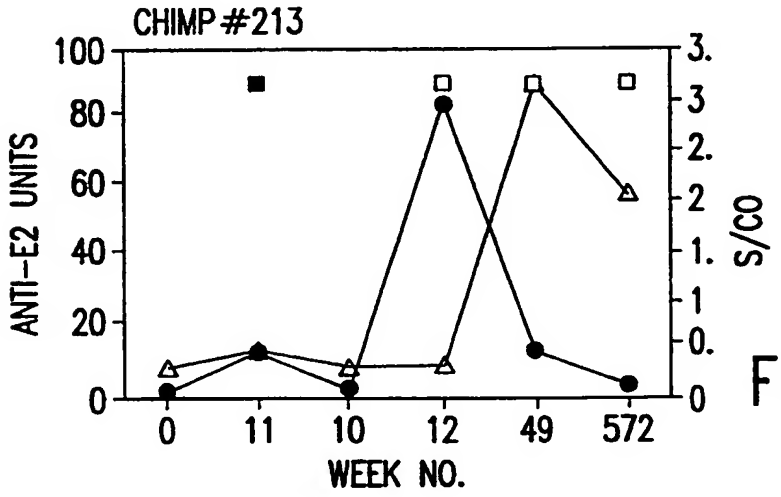

FIG.2B

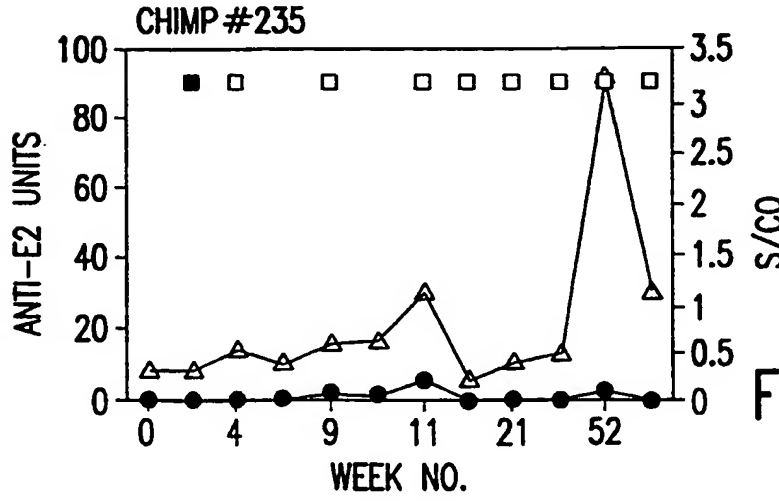

FIG.2C

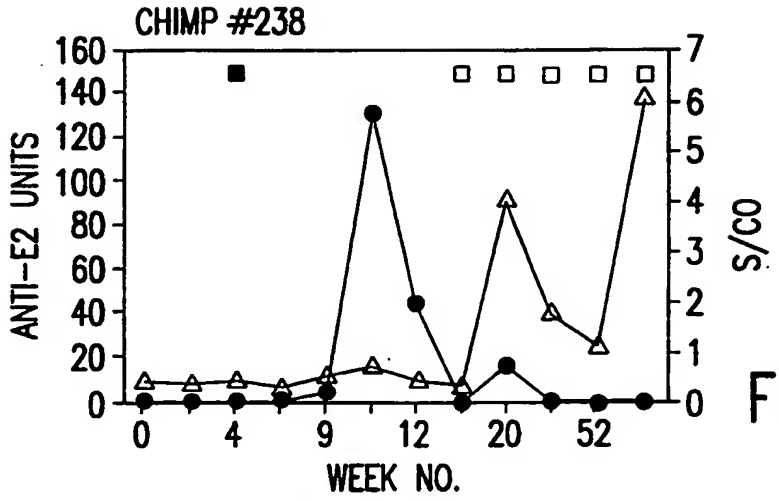

FIG.2D

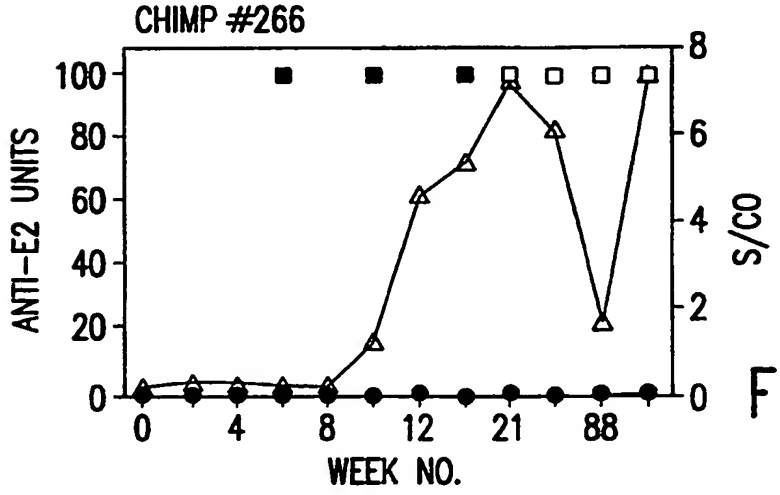

FIG.2E

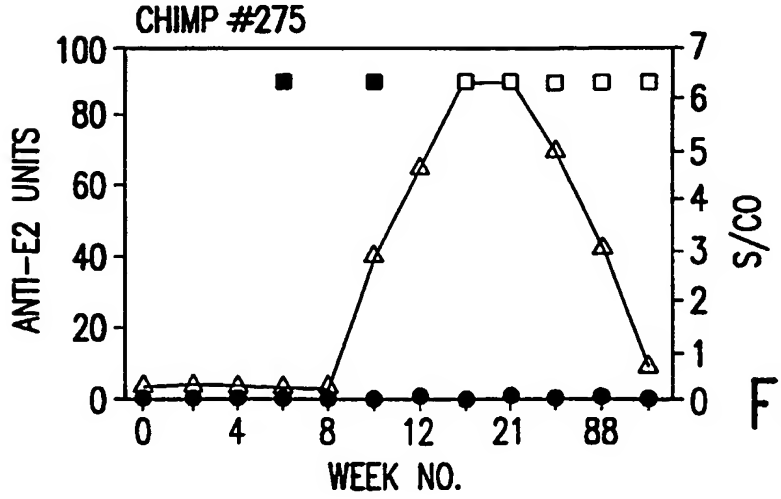

FIG.2F

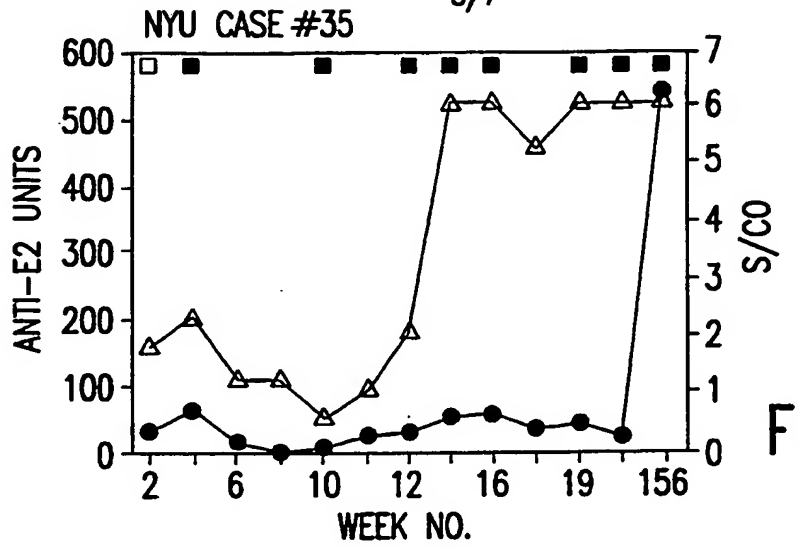

FIG.3A

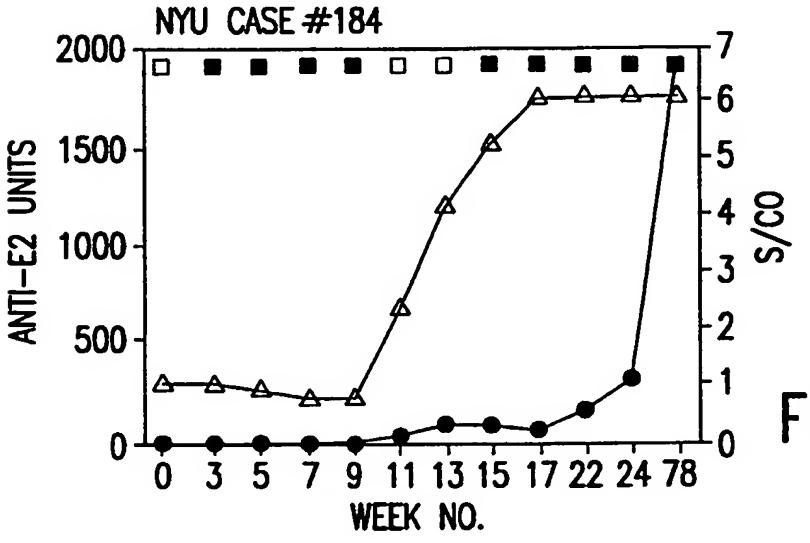

FIG.3B

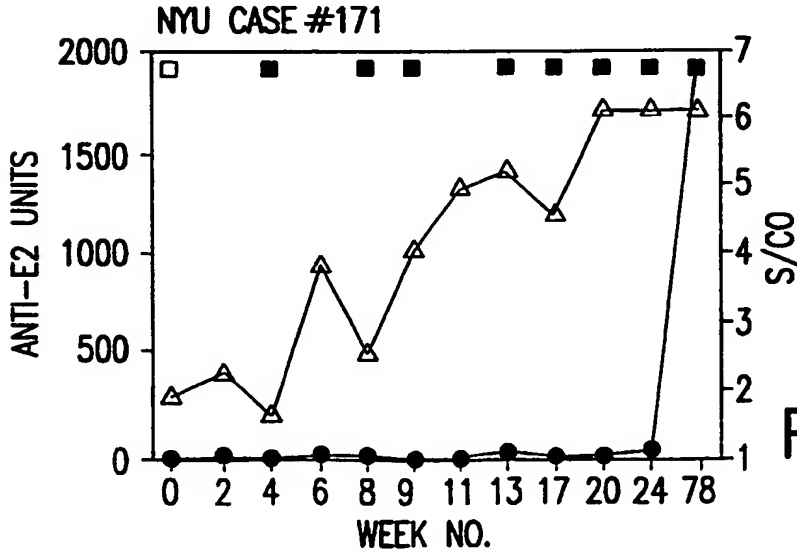

FIG.3C

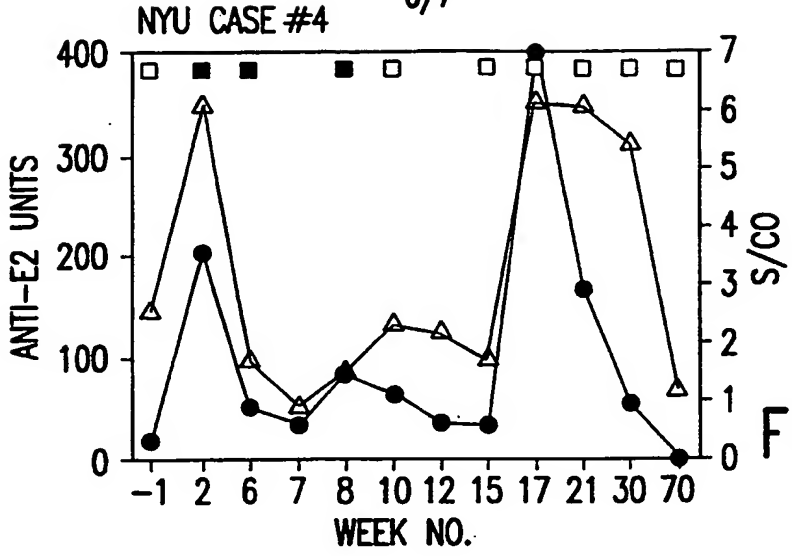

FIG.4A

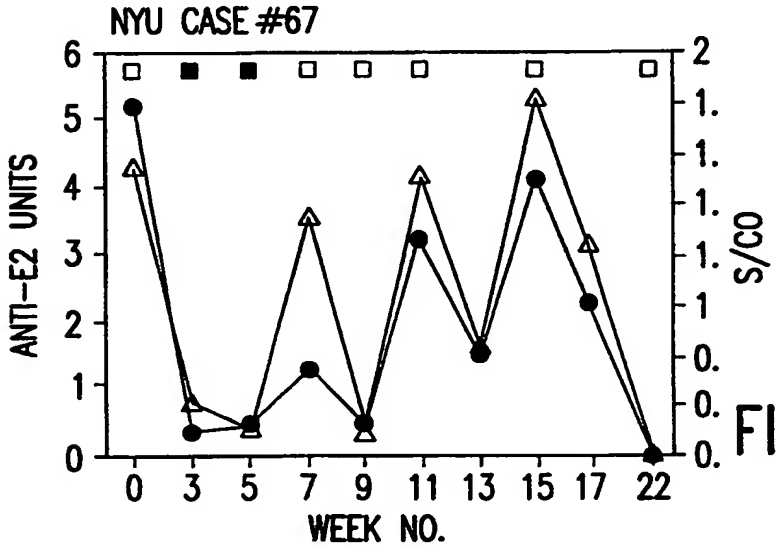

FIG.4B

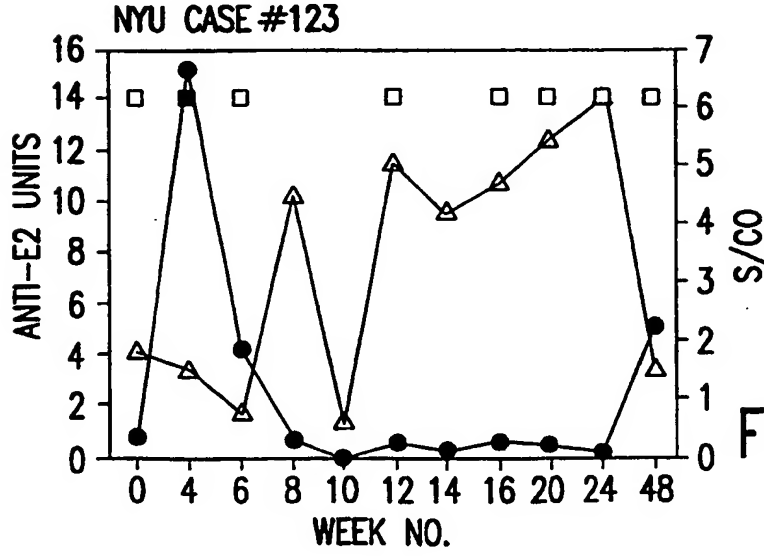

FIG.4C

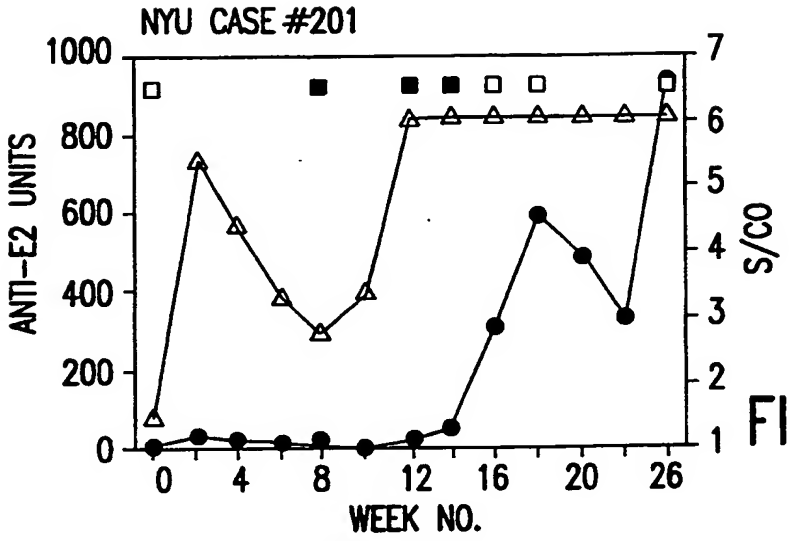
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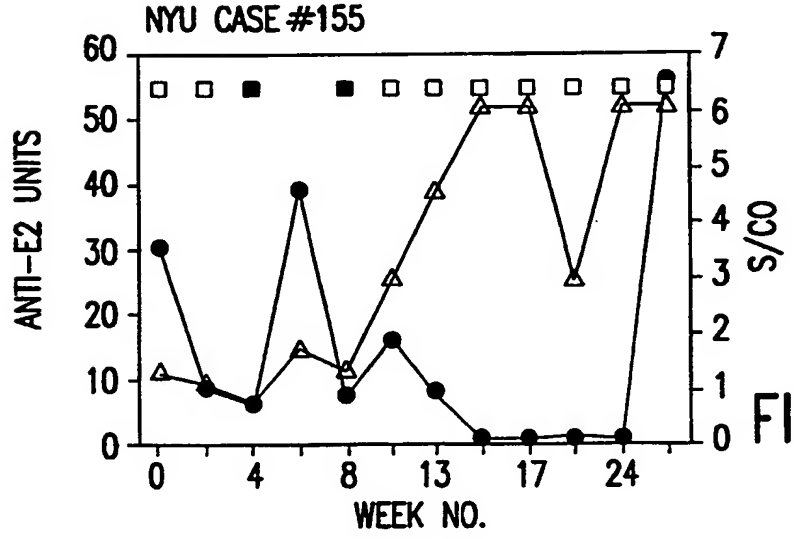
FIG.4D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WEEK NO.

FIG.4E

INTERNATIONAL SEARCH REPORT

Int. donal Application No
PCT/US 99/25254

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 G01N33/576

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 G01N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 98 16647 A (CHEMO SERO THERAPEUT RES INST ;NISHIHARA TSUKASA (JP); MIZUNO KYOS) 23 April 1998 (1998-04-23)	1-6,8
A	the whole document	7
X	WO 94 01778 A (CHIRON CORP) 20 January 1994 (1994-01-20) page 25, line 8 - line 11; claims 1,4; table 3	1,2,5,6, 8
X	WO 96 04300 A (US HEALTH) 15 February 1996 (1996-02-15) the whole document	1,2,5,6, 8
X	WO 93 06247 A (ABBOTT LAB) 1 April 1993 (1993-04-01) claim 1; figure 2 page 25, line 8 -page 26, line 14	1,2,5,6, 8

☐ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

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"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
8 February 2000	15/02/2000

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL – 2280 HV Rijswijk Tel. (+31–70) 340–2040, Tx. 31 651 epo nl, Fax: (+31–70) 340–3016	Gundlach, B

1

Form PCT/ISA/210 (second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

Information on patent family members

Int ational Application No
PCT/US 99/25254

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